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CHO, BHK, 293 cells, Vero expressed  
interferon alpha-14C.

h = 1 to 3;  
a-g, j-m, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
n, v-y = 0; z = 1.

↓  
1. CMP-SA-PEG,  $\alpha$ 2,8-ST

h = 1 to 3;  
a-g, i, r-u (independently selected) = 0 or 1;  
j-m (independently selected) = 0 to 2;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 2;  
z = 1; n = 0; R = PEG.

FIG. 30J

CHO, BHK, 293 cells, Vero expressed  
Interferon alpha-14C.  
a-g, j-m, r-u (independently selected) = 0 or 1;  
h = 1 to 3; n, v-y = 0; z = 1.

↓  
1. Sialidase  
2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1;  
h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 30K

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CHO, BHK, 293 cells, Vero expressed  
interferon alpha-14C.

h = 1 to 3;

a-g, j-m, i (independently selected) = 0 or 1;

r-u (independently selected) = 0 or 1;

n, v-y = 0; z = 1.

↓  
1. CMP-SA,  $\alpha$ 2,8-ST

h = 1 to 3;

a-g, i, r-u (independently selected) = 0 or 1;

j-m (independently selected) = 0 to 40;

z = 1; v-y, n = 0.

## FIG. 30L

Insect cell or fungi expressed interferon alpha-14C.

a-d, f, h, j-n, s, u, v-y = 0;

e, g, i, r, t (independently selected) = 0 or 1;

z = 1.

↓  
1. GNT's 1 & 2, UDP-GlcNAc

2. Galactosyltransferase,  
UDP-Gal-linker-SA-CMP

3. ST3Gal3, transferrin

a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1;

z = 1; b, d, f, h, j-n, s, u, w, y = 0;

R = transferrin.

## FIG. 30M

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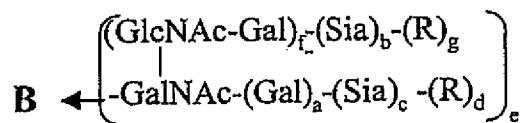
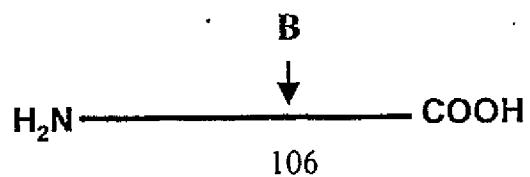
Insect cell or fungi expressed interferon alpha-14C.  
a-d, f, h, j-n, s, u, v-y = 0;  
e, g, i, r, t (independently selected) = 0 or 1; z = 1.

- ↓
1. endoglycanase
  2. Galactosyltransferase,  
    UDP-Gal-linker-SA-CMP
  3. ST3Gal3, transferrin

i (independently selected) = 0 or 1;  
a-h, j-m, r-z = 0;  
n = 1; R' = -Gal-linker-transferrin.

FIG. 30N

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a-c, e, f (independently selected) = 0 or 1;  
d, g = 0; R = polymer, glycoconjugate.

FIG. 30O

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CHO, BHK, 293 cells, Vero expressed  
IF-alpha (2a or 2b).  
a-c (independently selected) = 0 or 1;  
e = 1; d, f, g = 0

- ↓  
1. Sialidase  
2. CMP-SA-PEG, ST3Gal1

a-d (independently selected) = 0 or 1;  
e = 1; b, f, g = 0; R = PEG.

FIG. 30P

Insect cell expressed interferon alpha (2a or 2b).  
a, e (independently selected) = 0 or 1;  
b, c, d, f, g = 0.

- ↓  
1. Galactosyltransferase, UDP-Gal  
2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1;  
b, f, g = 0; R = PEG.

FIG. 30Q

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E. coli expressed IF-alpha (2a or 2b).  
a-g = 0.

↓  
1. GalNAc Transferase,  
UDP-GalNAc-PEG

a-c, f, g = 0; d, e = 1; R = PEG.

FIG. 30R

NSO expressed IF-alpha (2a or 2b).  
a (independently selected) = 0 or 1;  
e = 1; b, c, d, f, g = 0

↓  
1. CMP-SA-levulinate, ST3Gal1  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a, c, d (independently selected) = 0 or 1;  
e = 1; b, f, g = 0; R = PEG.

FIG. 30S

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E. coli expressed IF-alpha (2a or 2b).  
a-g = 0.

↓  
1. Endo-N-acetylgalatosamidase  
(synthetic enzyme),  
PEG-Gal-GalNAc-F

a, d, e = 1; b, c, f, g = 0; R = PEG.

FIG. 30T

E. coli expressed IF-alpha (2a or 2b).  
a-g = 0.

↓  
1. GaINAc Transferase, UDP-GalNAc  
2. sialyltransferase, CMP-SA-PEG

b, d = 0 or 1; e = 1; a, c, f, g = 0; R = PEG.

FIG. 30U

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CHO, BHK, 293 cells, Vero expressed IF-alpha  
(2a or 2b).  
a-c, f (independently selected) = 0 or 1;  
e = 1; d, g = 0

- ↓  
1. Sialidase  
2. CMP-SA-PEG, ST3Gal1 and ST3Gal3

a-d, f, g (independently selected) = 0 or 1;  
e = 1; R = PEG.

FIG. 30V

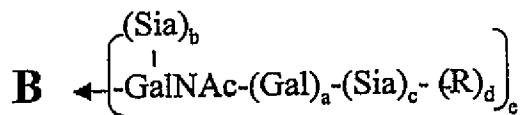
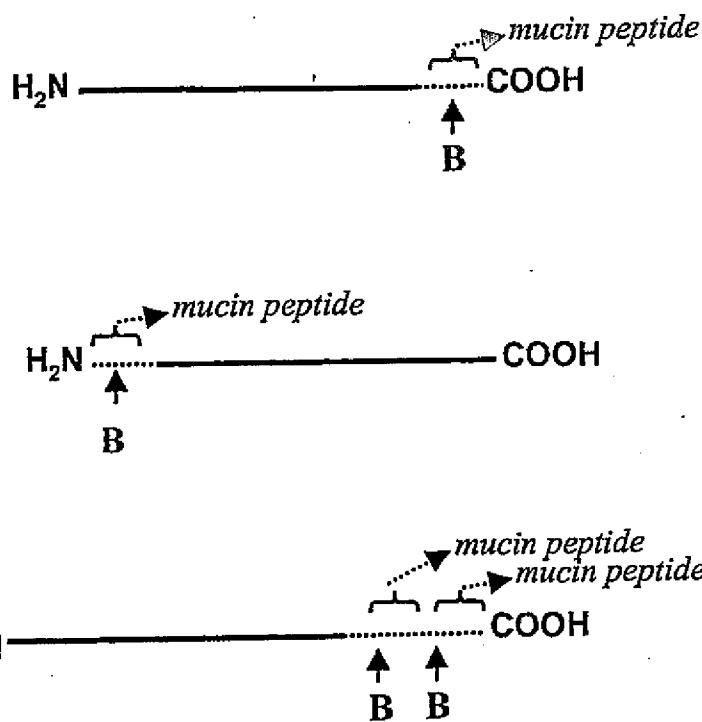
CHO, BHK, 293 cells, Vero expressed  
IF-alpha (2a or 2b).  
a-c, f (independently selected) = 0 or 1;  
e = 1; d, g = 0

- ↓  
1. Sialidase  
2. CMP-SA-linker-SA-CMP,  
,ST3Gal1  
3. ST3Gal3, transferrin

a-d, f (independently selected) = 0 or 1;  
e = 1; R = transferrin; g = 0.

FIG. 30W

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$a-c, e$  (independently selected) = 0 or 1;  
 $d = 0$ ;  $R$  = polymer, glycoconjugate.

FIG. 30X

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CHO, BHK, 293 cells, Vero expressed  
interferon alpha-mucin fusion protein.  
a-c, e (independently selected) = 0 or 1; d = 0

- ↓  
1. Sialidase  
2. CMP-SA-PEG, ST3Gall

a-d, e (independently selected) = 0 or 1;  
R = PEG.

FIG. 30Y

Insect cell expressed interferon alpha-mucin  
fusion protein.

a, e (independently selected) = 0 or 1;  
b, c, d = 0.

- ↓  
1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1;  
b, c = 0; R = PEG.

FIG. 30Z

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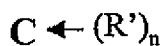
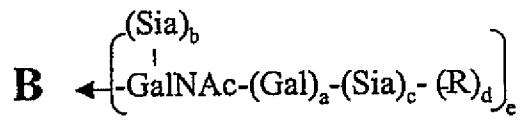
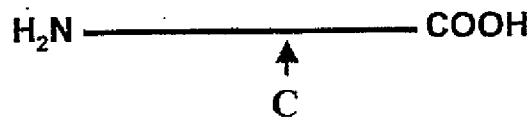
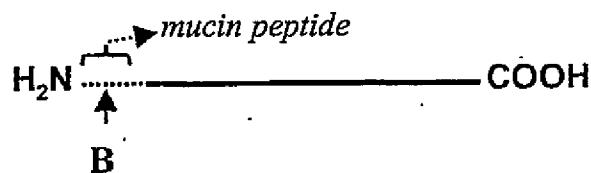
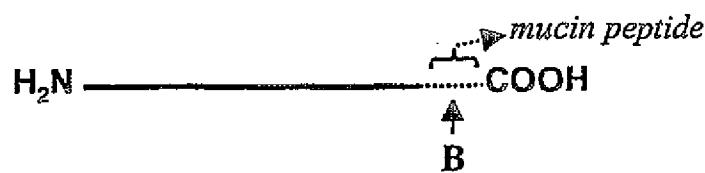
E. coli expressed interferon alpha-mucin fusion protein.  
a-e = 0.

- ↓  
1. GalNAc Transferase, UDP-GalNAc  
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;  
a, b = 0; R = PEG.

FIG. 30AA

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a-c, e (independently selected) = 0 or 1;  
 d = 0; R = polymer, linker.

(

**FIG. 30BB**

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E. coli expressed interferon alpha-mucin fusion protein.  
a-e, n = 0.

↓  
1. GalNAc Transferase,  
UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1;  
a-c, n = 0; R = PEG.

FIG. 30CC

E. coli expressed interferon alpha-mucin fusion protein.  
a-e, n = 0.

↓  
1. GalNAc Transferase,  
UDP-GalNAc-linker-SA-CMP  
2. ST3Gal3, asialo-transferrin  
3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1;  
a-c, n = 0; R = linker-transferrin.

FIG. 30DD

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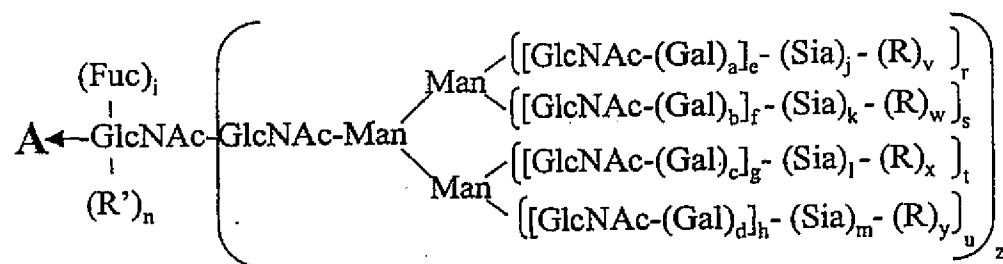
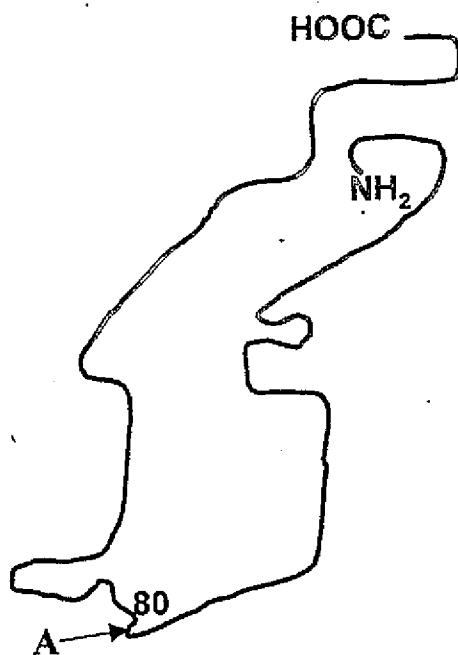
E. coli expressed Interferon alpha (no fusion).  
a-e, n = 0.

1. NHS-CO-linker-SA-CMP  
2. ST3Gal3, transferrin

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 30EE

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a-d, i, r-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 or 1.

n, v-y = 0; z = 0 or 1; R = polymer

**FIG. 31A**

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CHO, BHK, 293 cells, Vero expressed IF-beta  
 $h = 1 \text{ to } 3;$   
 $a-g, j-m, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $n, v-y = 0; z = 1.$

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3

$h = 1 \text{ to } 3;$   
 $a-g, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $j-m, v-y$  (independently selected) = 0 or 1;  
 $z = 1; n = 0; R = PEG.$

FIG. 31B

Insect cell expressed IF-beta  
 $a-d, f, h, j-n, s, u, v-y = 0;$   
 $e, g, i, r, t$  (independently selected) = 0 or 1;  
 $z = 1.$

- ↓  
 1. GNT's 1&2, UDP-GlcNAc  
 2. Galactosyltransferase, UDP-Gal  
 2. CMP-SA-PEG, ST3Gal3,  
 buffer, salt

$b, d, f, h, k, m, n, s, u, w, y = 0;$   
 $a, c, e, g, i, r, t$  (independently selected) = 0 or 1;  
 $j, l, v, x$  (independently selected) = 0 or 1;  
 $z = 1; R = PEG.$

FIG. 31C

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Yeast expressed IF-beta  
 $a-n = 0; z = 1;$   
 $r-y$  (independently selected) = 0 to 1;  
 $R$  (branched or linear) = Man, oligomannose or  
polysaccharide.

- ↓
1. Endo-H
  2. Galactosyltransferase, UDP-Gal
  - 3.. CMP-SA-PEG, ST3Gal3

$a-m, r-z= 0; n = 1; R' = -Gal-Sia-PEG.$

FIG. 31D

CHO, BHK, 293 cells, Vero expressed IF-beta  
 $h = 1$  to 3;  
 $a-g, j-m, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $n, v-y = 0; z = 1.$

- ↓
1. CMP-SA-PEG, ST3Gal3

$h = 1$  to 3;  
 $a-g, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $j-m, v-y$  (independently selected) = 0 or 1;  
 $z = 1; n = 0; R = PEG.$

FIG. 31E

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Insect cell expressed IF-beta  
a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t  
(independently selected) = 0 or 1; z = 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

## FIG. 31F

Yeast expressed IF-beta  
a-n = 0; z = 1;  
r-y (independently selected) = 0 to 1;  
R (branched or linear) = Man, oligomannose.

- ↓  
1. mannosidases  
2. GNT's 1,2,4,5, UDP-GlcNAc  
3. Galactosyltransferase, UDP-Gal  
4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

## FIG. 31G

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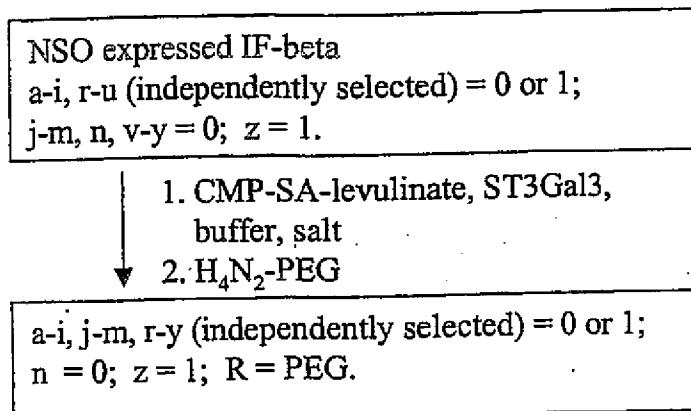


FIG. 31H

CHO, BHK, 293 cells, Vero expressed IF-beta  
h = 1 to 3;  
a-g, j-m, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
n, v-y = 0; z = 1.

↓  
1. CMP-SA-PEG, α2,8-ST

h = 1 to 3;  
a-g, i, r-u (independently selected) = 0 or 1;  
j-m (independently selected) = 0 to 2;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 2;  
z = 1; n = 0; R = PEG.

FIG. 31I

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CHO, BHK, 293 cells, Vero expressed IF-beta  
a-g, j-m, r-u (independently selected) = 0 or 1;  
h = 1 to 3; n, v-y = 0; z = 1.

- ↓  
1. Sialidase  
2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1;  
h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 31J

CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n=0;  
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31K

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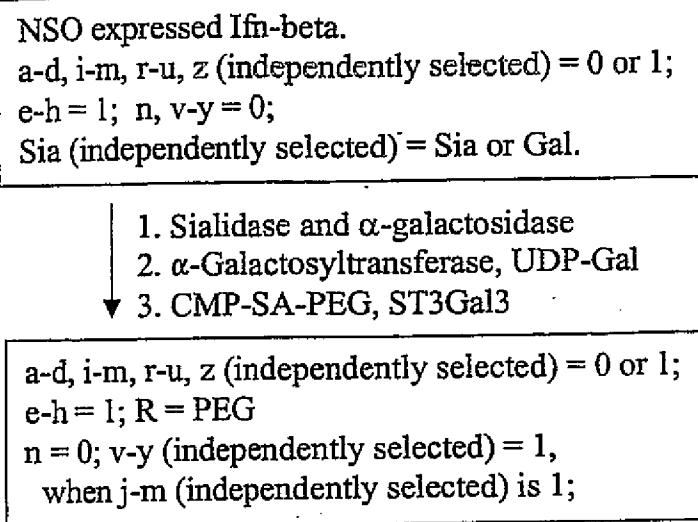


FIG. 31L

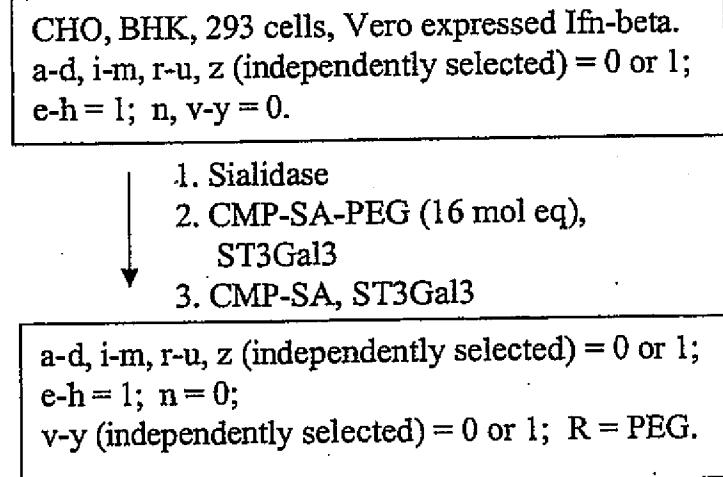


FIG. 31M

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CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓  
1. CMP-SA-levulinic acid, ST3Gal3,  
buffer, salt  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n = 0;  
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31N

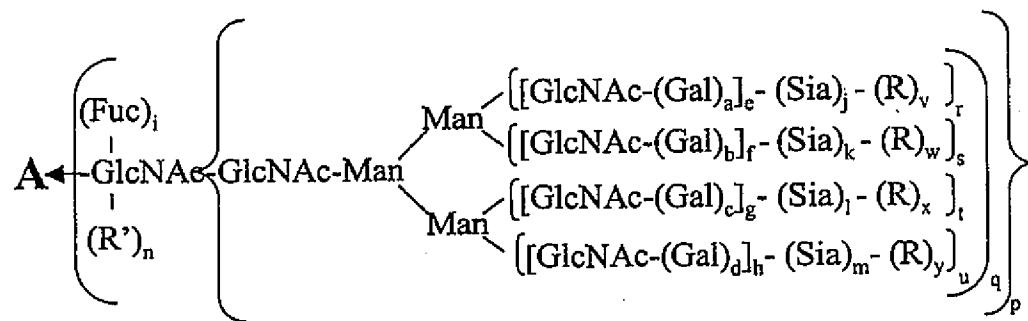
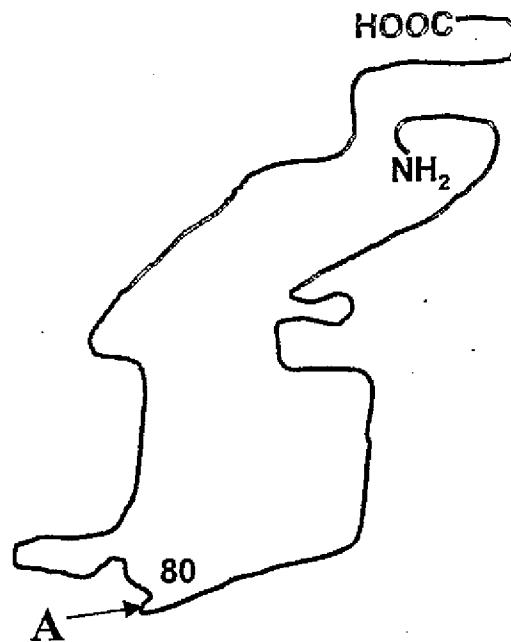
CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓  
1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
n, v-y (independently selected) = 0.

FIG. 31O

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a-d, i, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = modifying group;

R' = H, glycosyl group, modifying group,  
glycoconjugate.

FIG. 31P

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Insect cell expressed Ifn-beta.  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

## FIG. 31Q

Yeast expressed Ifn-beta.  
a-m = 0; q-y (independently selected) = 0 to 1;  
p = 1;  
R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0;  
n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

## FIG. 31R

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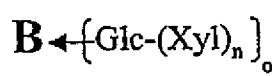
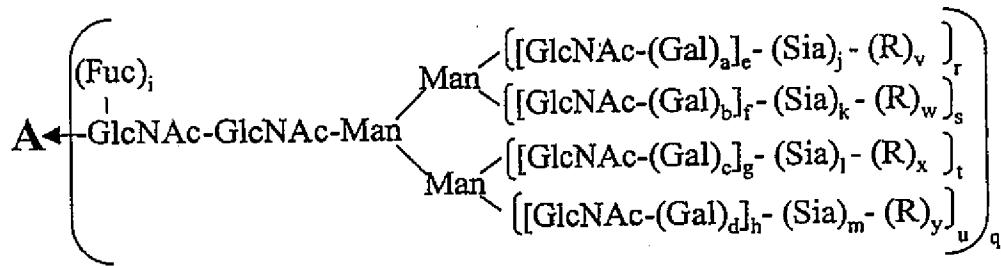
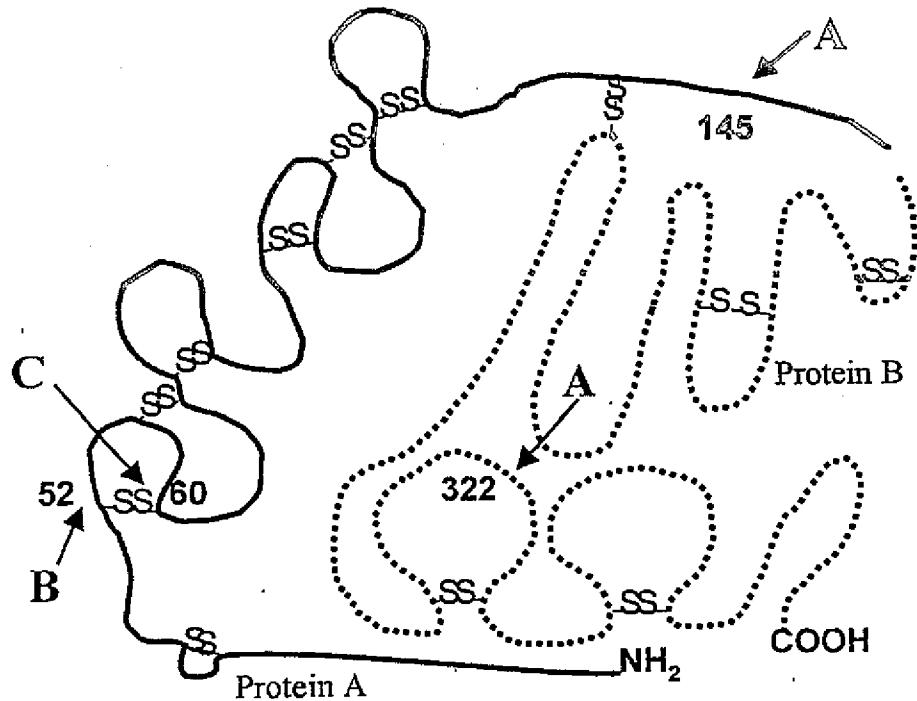
CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

1. CMP-SA-linker-SA-CMP,  
ST3Gal3
2. ST3Gal3, desialylated transferrin.
3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 31S

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a-d, i, q-u (independently selected) = 0 or 1.

o, p (independently selected) = 0 or 1.

e-h, n (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-y = 0;

R = modifying group, mannose, oligo-mannose, Sia-Lewis X, Sia-Lewis A..

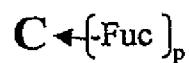


FIG. 32A

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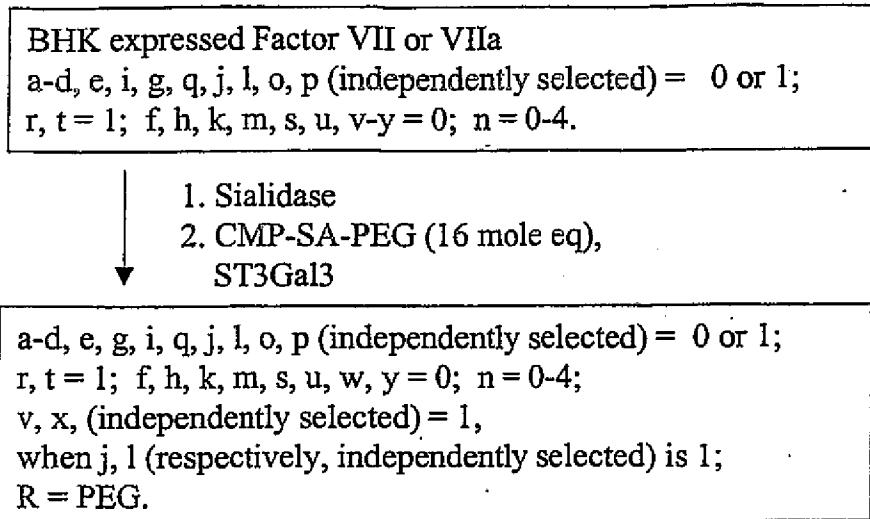


FIG. 32B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa  
 a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- ↓
1. Sialidase  
 2. CMP-SA-PEG (1.2 mole eq),  
 ST3Gal3  
 3. CMP-SA (8 mol eq), ST3Gal3

a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;  
 v or x, (independently selected) = 1,  
 when j or l, (respectively, independently selected) is 1;  
 R = PEG.

FIG. 32C

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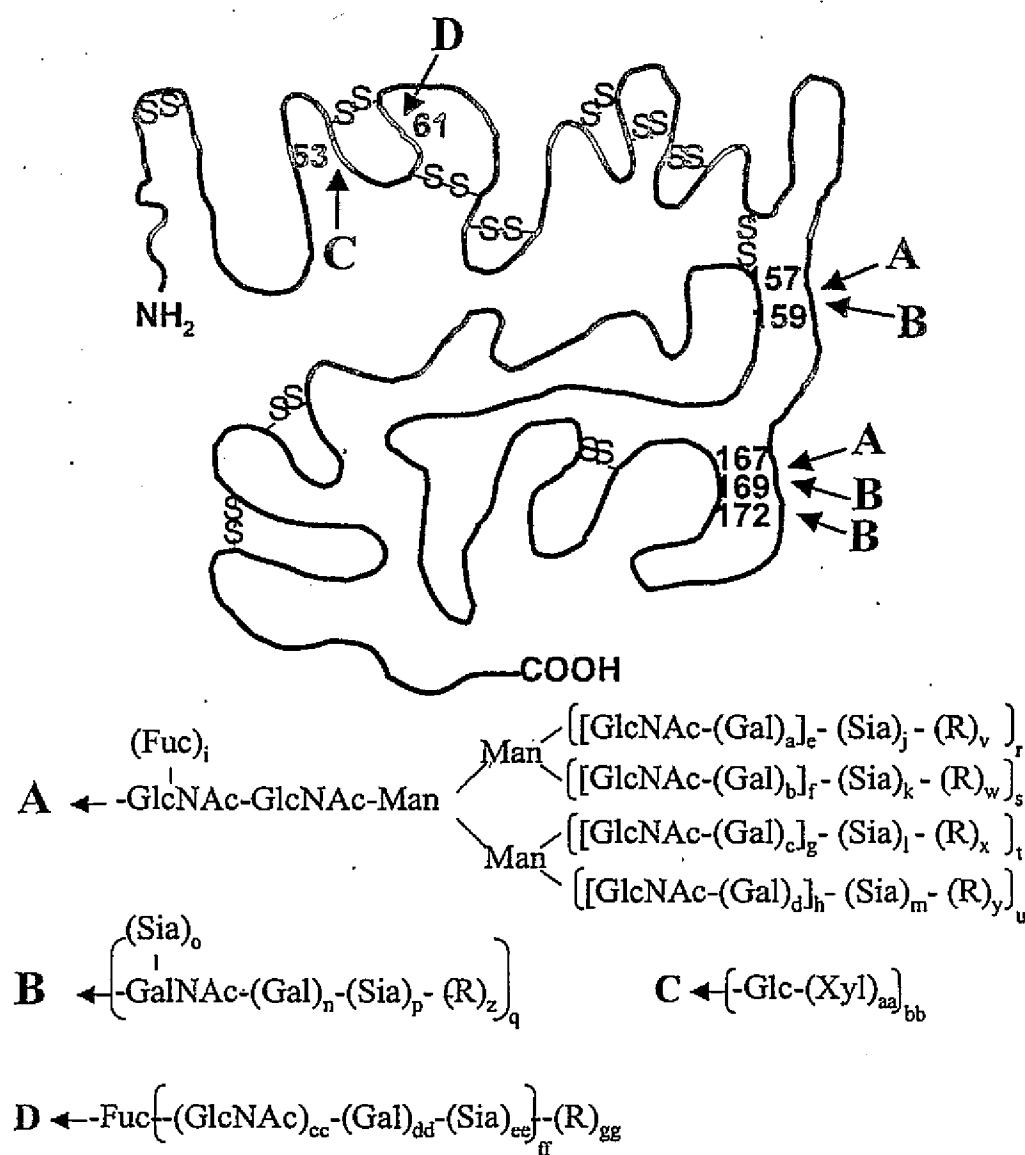
NSO expressed Factor VII or VIIa  
a-u (independently selected) = 0 or 1;  
v-y = 0; n = 0-4;  
Sia (independently selected) = Sia or Gal.

- ↓  
1. Sialidase and  $\alpha$ -galactosidase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, o-u (independently selected) = 0 or 1;  
n = 0-4; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
Sia = Sia; R = PEG.

FIG. 32D

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a-d, i, n-u (independently selected) = 0 or 1.

bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.

e-h, aa (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 33A

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CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1;  
 R = PEG.

FIG. 33B

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3  
 3. ST3Gal1, CMP-SA

a-d, n, p, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1;  
 o, z = 0; R = PEG.

FIG. 33C

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CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i,  
 o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- ↓
1. sialidase
  2. Galactosyltransferase, UDP-Gal
  3. CMP-SA, ST3Gal3
  4. CMP-SA-PEG, ST3Gal1

a-d, n, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =  
 0 or 1; R = PEG;  
 o, v-y, gg = 0;  
 j-m, p, ee (independently selected) = 0 or 1, but when  
 p = 1, z = 1.

**FIG. 33D**

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently  
 selected) = 0 or 1;  
 v-z, gg = 0.

↓

CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)  
 = 0 or 1; R = PEG;  
 o, p, z = 0; j-m, ee, v-y, gg (independently selected) =  
 0 or 1.

**FIG. 33E**

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CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0; R = PEG;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1.

FIG. 33F

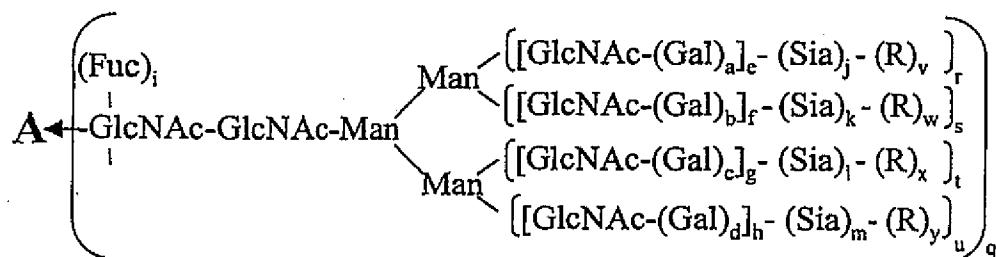
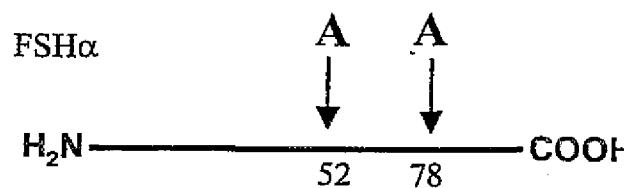
CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q, bb, cc, dd, ff = 1;  
 e-h, aa = 1 to 4;  
 ee, j-m, i, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

1. CMP-SA-PEG,  $\alpha$ 2,8-ST

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0; R = PEG;  
 j-m, ee (independently selected) = 0 to 2;  
 v-y, gg (independently selected) = 1, when j-m (independently selected) is 2;

FIG. 33G

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a-d, i, q-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose.

FIG. 34A

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CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 34B

CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 34C

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NSO expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0;  
Sia (independently selected) = Sia or Gal.

- ↓  
1. Sialidase and  $\alpha$ -galactosidase  
2. Galactosyltransferase, UDP-Gal  
↓ 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 34D

CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3  
↓ 3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 34E

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CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 34F

CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 34G

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Insect cell expressed FSH.  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

FIG. 34H

Yeast expressed FSH.  
a-m = 0; q-y (independently selected) = 0 to 1;  
p = 1;  
R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0;  
n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

FIG. 34I

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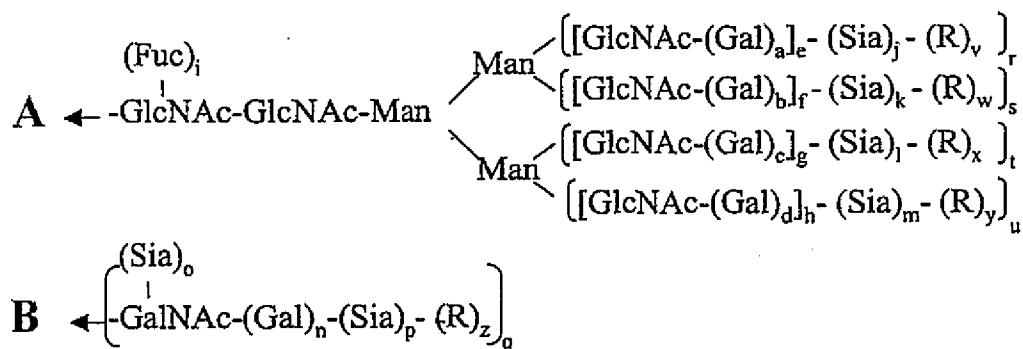
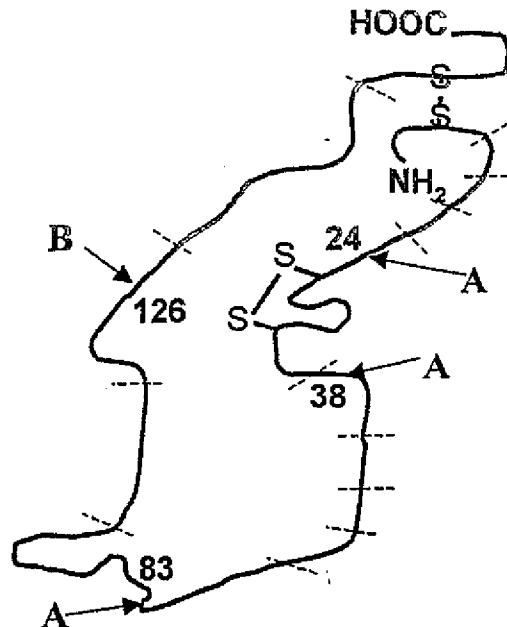
CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP, ST3Gal3
  2. ST3Gal1, desialylated chorionic  
gonadotrophin (CG) produced in CHO.
  3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-CG.

FIG. 34J

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

v-z = 0;

R = polymer.

FIG. 35A

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CHO, BHK, 293 cells, Vero expressed EPO  
 a-g, n, q = 1; h = 1 to 3;  
 j-m, i, o, p (independently selected) = 0 or 1;  
 r-u (independently selected) = 0 to 1; v-z = 0

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3

a-g, n, q = 1; h = 1 to 3;  
 i, o, p (independently selected) = 0 or 1;  
 r-u (independently selected) = 0 or 1;  
 j-m, v-y (independently selected) = 0 or 1;  
 R = PEG; z = 0.

FIG. 35B

Insect cell expressed EPO  
 a-d, f, h, j-q, s, u, v-z = 0;  
 e, g, i, r, t (independently selected) = 0 or 1.

- ↓  
 1. GNT's 1&2, UDP-GlcNAc  
 2. Galactosyltransferase, UDP-Gal  
 2. CMP-SA-PEG, ST3Gal3

b, d, f, h, k, m-q, s, u, w, y, z = 0;  
 a, c, e, g, i, r, t (independently selected) = 0 or 1;  
 j, l, v, x (independently selected) = 0 or 1;  
 R = PEG.

FIG. 35C

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CHO, BHK, 293 cells, Vero expressed EPO  
a-q, r-u (independently selected) = 0 or 1;  
v-z = 0.

1. sialidase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA, ST3Gal3
4. CMP-SA-PEG, ST3Gal1

a-h, n, q = 1;  
i-m, o, r-u (independently selected) = 0 or 1;  
v-y = 0; p, z = 0 or 1; R = PEG.

FIG. 35D

CHO, BHK, 293 cells, Vero expressed EPO  
a-g, n, q = 1; h = 1 to 3;  
j-m, i, o, p (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
v-z = 0

1. CMP-SA-PEG, ST3Gal3

a-g, n, q = 1; h = 1 to 3;  
i, o, p (independently selected) = 0 or 1;  
r-u (independently selected) = 0 to 1;  
j-m, v-y (independently selected) = 0 or 1;  
R = PEG; z = 0.

FIG. 35E

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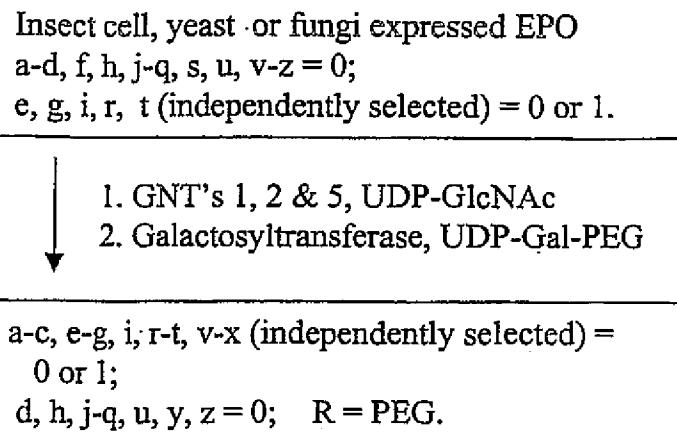


FIG. 35F

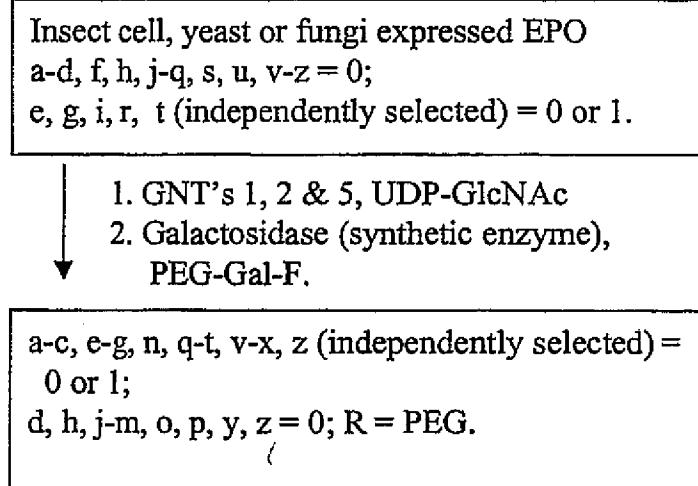


FIG. 35G

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Insect cell, yeast or fungi expressed EPO  
a-d, f, h, j-m, n-q, s, u, v-z = 0;  
e, g, i, r, t (independently selected) = 0 or 1.

↓  
1. GNT-1, UDP-GlcNAc-PEG

e, i, r, v (independently selected) = 0 or 1;  
a-h, j-q, s-u, w-z = 0; R = PEG.

## FIG. 35H

Insect cell, yeast or fungi expressed EPO  
a-d, f, h, j-m, n-q, s, u, v-z = 0;  
e, g, i, r, t (independently selected) = 0 or 1.

↓  
1. GNT-1, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a, e, i, r, v (independently selected) = 0 or 1;  
b-d, f-h, j-q, s-u, w-z = 0; R = PEG.

## FIG. 35I

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Insect cell, yeast or fungi expressed EPO  
a-d, f, h, j-m, n-q, s, u, v-z = 0;  
e, g, i, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT-1, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal  
3. ST3Gal3, CMP-SA-PEG

a, e, i, j, r, v (independently selected) = 0 or 1;  
b-d, f-h, k-q, s-u, w-z = 0; R = PEG.

FIG. 35J

Insect cell, yeast or fungi expressed EPO  
a-d, f, h, j-m, n-q, s, u, v-z = 0;  
e, g, i, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1, 2 & 5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal  
3. ST3Gal3, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected)  
= 0 or 1;  
d, h, m-q, u, y, z = 0; R = PEG.

FIG. 35K

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Insect cell, yeast or fungi expressed EPO  
a-d, f, h, j-m, n-q, s, u, v-z = 0;  
e, g, i, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1, 2 & 5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal  
3. α2,6-sialyltransferase, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected)  
= 0 or 1;  
d, h, m-q, u, y, z = 0; R = PEG.

## FIG. 35L

CHO, BHK, 293 cells, Vero expressed EPO  
a-q, r-u (independently selected) = 0 or 1;  
v-z = 0.

- ↓  
1. sialidase  
2. CMP-SA, ST3Gal3  
3. CMP-SA-PEG, ST3Gal1

a-h, q, i-o, r-u (independently selected)  
= 0 or 1;  
v-y = 0; p, z = 0 or 1; R = PEG.

## FIG. 35M

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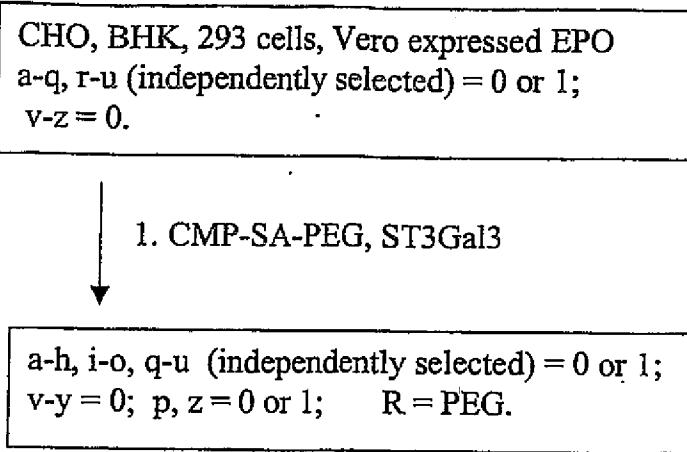


FIG. 35N

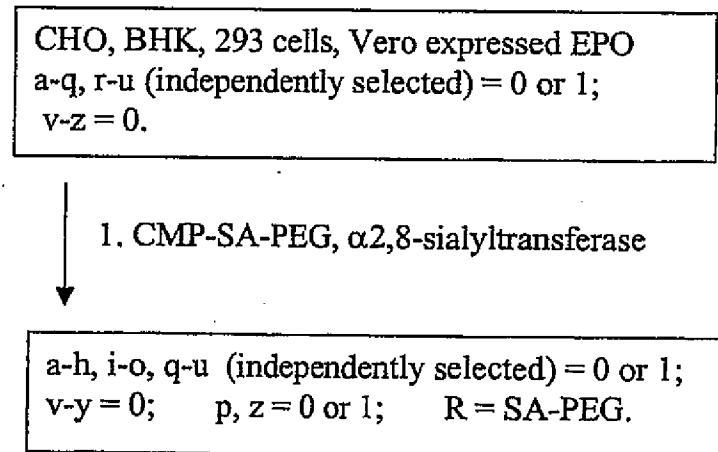


FIG. 35O

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CHO, BHK, 293 cells, Vero expressed EPO  
 $a-q, r-u$  (independently selected) = 0 or 1;  
 $v-z = 0$ .

↓  
 1. CMP-SA-PEG,  $\alpha 2,8$ -sialyltransferase

$a-h, i-o, p-u, v-z$  (independently selected)  
 = 0 or 1;  
 $R = SA-PEG$ .

## FIG. 35P

yeast or fungi expressed EPO  
 $r, t, u, v, x, y$  (independently selected) = 0 or 1;  
 $a-m, n-q, s, w, z = 0$ ;  $R = (Man)_n$   
 where  $n = 1-5$ , linear or branched.

↓  
 1. mannosidases  
 2. GNT-1, UDP-GlcNAc  
 3. galactosyltransferase, UDP-Gal  
 4. ST3Gal3, CMP-SA-PEG

$a, e, j, r, v$  (independently selected) = 0 or 1;  
 $b-d, f-i, k-q, s-u, w-z = 0$ ;  $R = PEG$ .

## FIG. 35Q

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yeast or fungi expressed EPO  
 $r, t, u, v, x, y$  (independently selected) = 0 or 1;  
 $a-m, n-q, s, w, z = 0$ ;  $R = (\text{Man})_n$   
 where  $n = 1-5$ , linear or branched.

- ↓  
 1. mannosidases  
 2. GNT-1, UDP-GlcNAc-PEG

$e, r, v$  (independently selected) = 0 or 1;  
 $a-h, i-q, s-u, w-z = 0$ ;  $R = \text{PEG}$ .

FIG. 35R

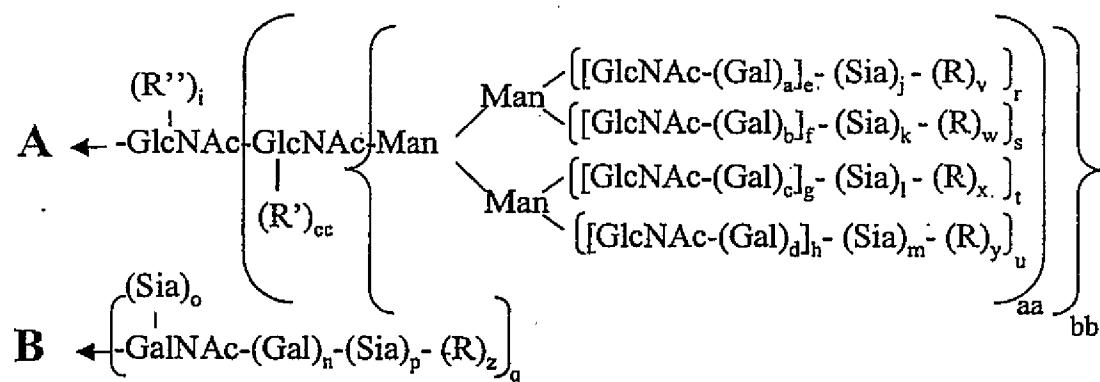
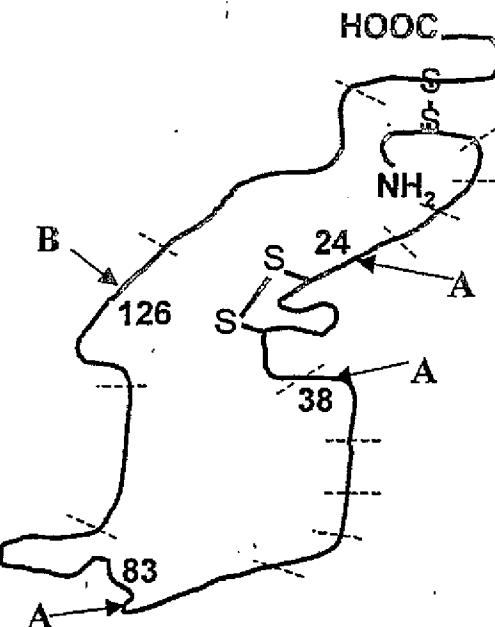
yeast or fungi expressed EPO  
 $r, t, u, v, x, y$  (independently selected) = 0 or 1;  
 $a-m, n-q, s, w, z = 0$ ;  $R = (\text{Man})_n$   
 where  $n = 1-5$ , linear or branched.

- ↓  
 1. mannosidase-I  
 2. GNT-1, UDP-GlcNAc  
 3. galactosyltransferase, UDP-Gal  
 4. ST3Gal3, CMP-SA-PEG

$a, e, j, r, t-u, v, x, y$  (independently selected)  
 = 0 or 1;  
 $b-d, f-i, k-q, s, w, z = 0$ ;  
 $(R)_v = \text{PEG}$ ;  $(R)_x$  and  $(R)_y = \text{Man}$ .

FIG. 35S

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

v-z = 0; aa, bb = 1; cc = 0;

R = polymer; R'' and R' = sugar-polymer or Fuc.

FIG. 35T

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yeast or fungi expressed EPO  
r, t, u, v, x, y (independently selected) = 0 or 1;  
cc, a-m, n-q, s, w, z = 0;  
aa, bb = 1;  
 $R = (\text{Man})_n$  where  $n = 1-100$ , linear or branched.

- ↓  
1. endo-H  
2. galactosyltransferase, UDP-Gal-PEG

i (independently selected) = 0 or 1;  
aa, bb, cc, a-h, j-z = 0;  $R'' = \text{Gal-PEG}$ .

## FIG. 35U

yeast or fungi expressed EPO  
r, t, u, v, x, y (independently selected) = 0 or 1;  
cc, a-m, n-q, s, w, z = 0; aa, bb = 1;  
 $R = (\text{Man})_n$  where  $n = 1-100$ , linear or branched.

- ↓  
1. endo-H  
2. galactosyltransferase, UDP-Gal  
3. ST3Gal3, CMP-SA-PEG

i (independently selected) = 0 or 1;  
aa, bb, cc, a-h, j-z = 0;  $R'' = \text{Gal-SA-PEG}$ .

## FIG. 35V

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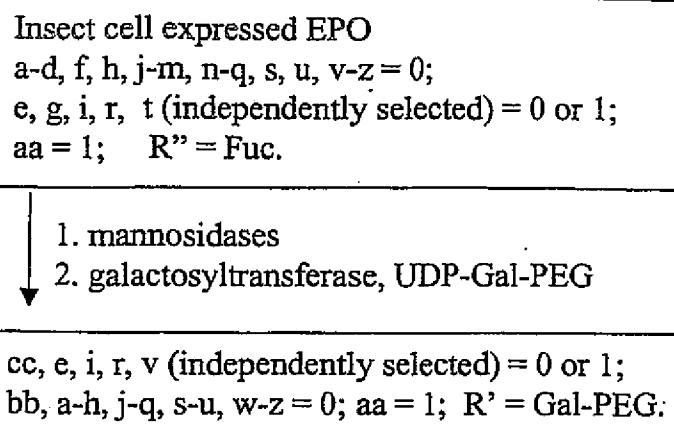
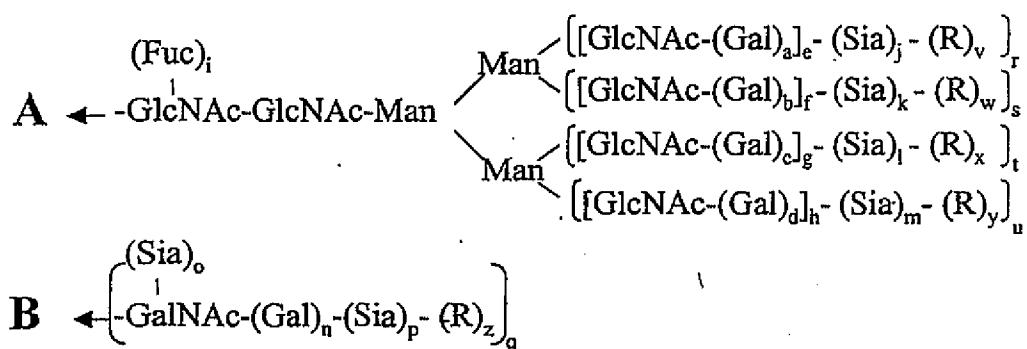
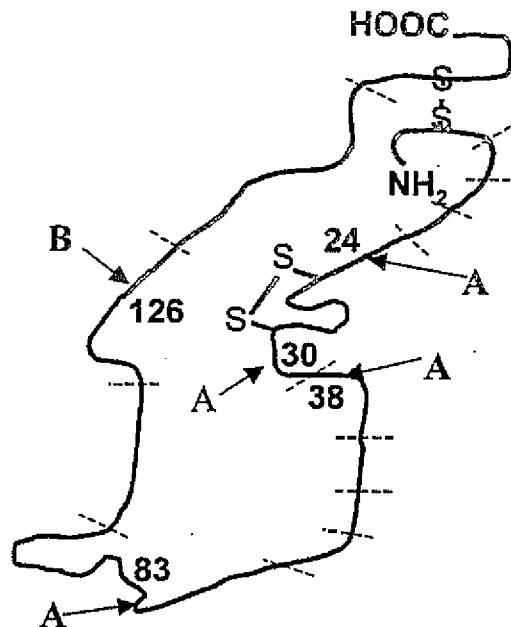


FIG. 35W

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

$$y-z=0$$

R = polymer.

FIG. 35X

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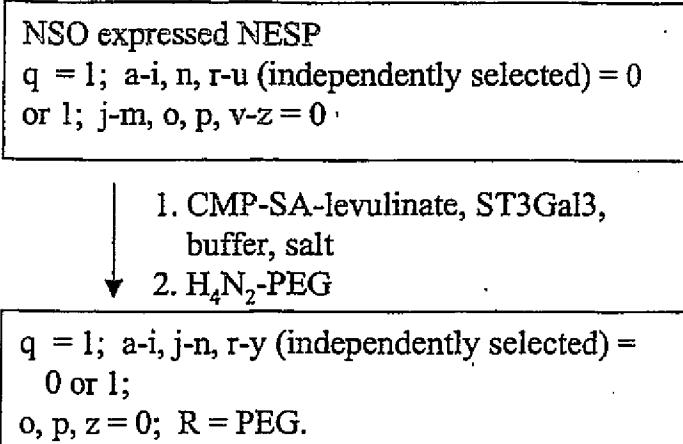


FIG. 35Y

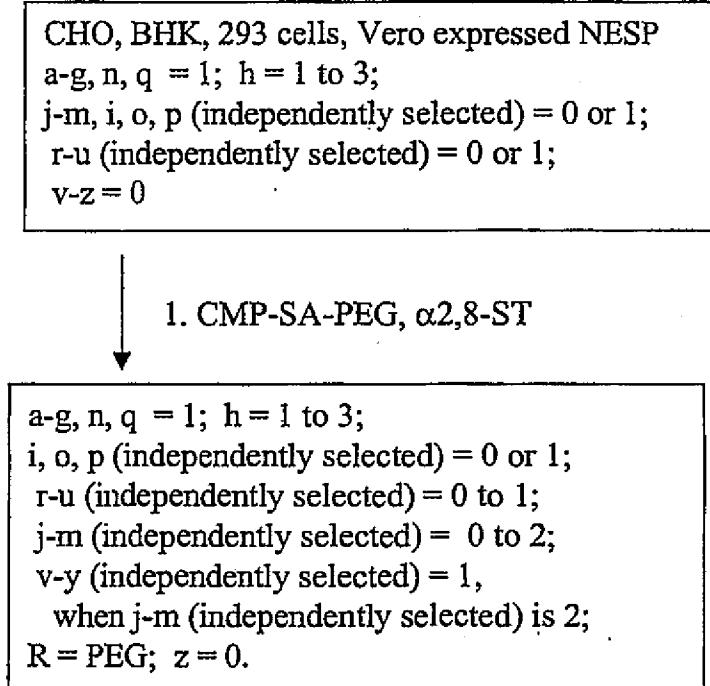


FIG. 35Z

123/497

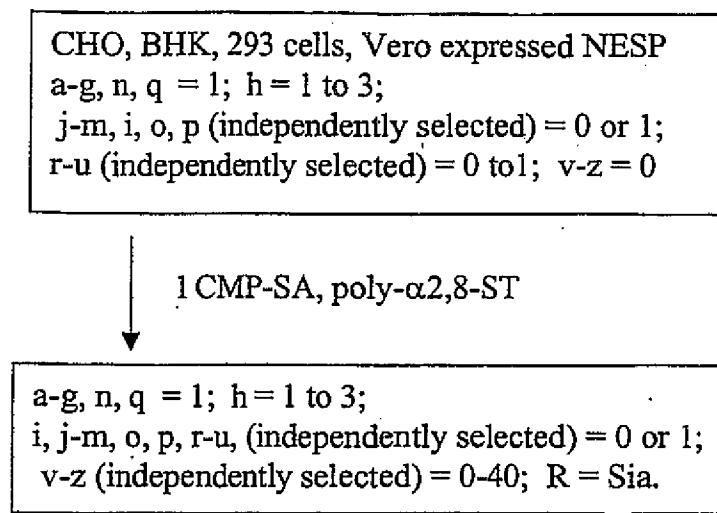
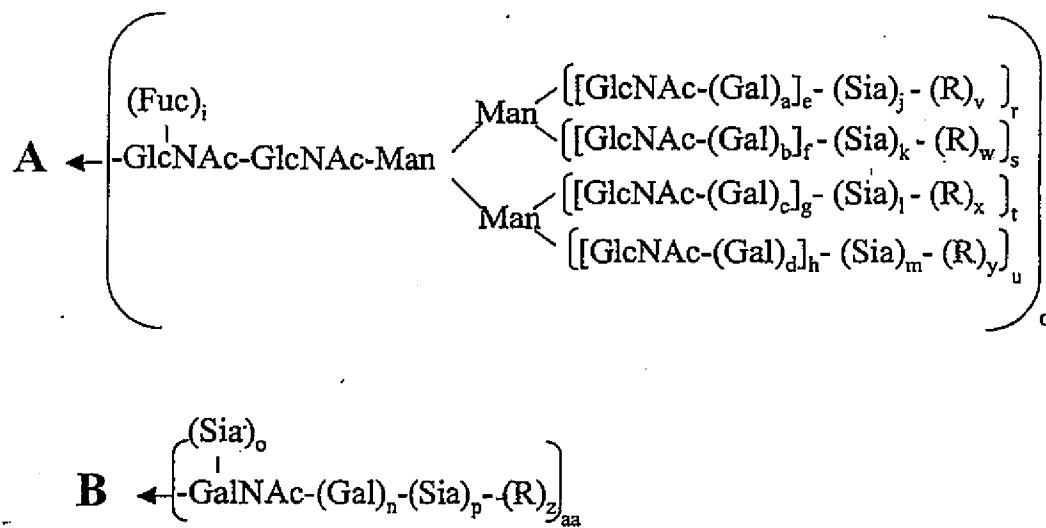
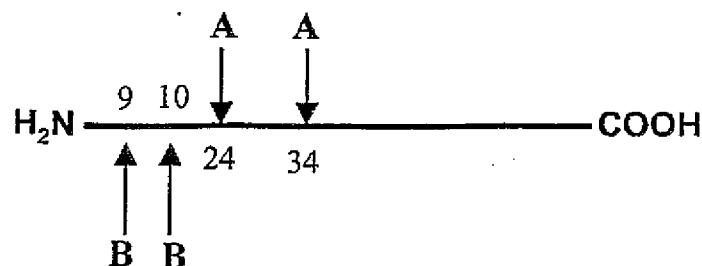


FIG. 35AA

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a-d, i, n-u, aa (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer, glycoconjugate.

FIG. 36A

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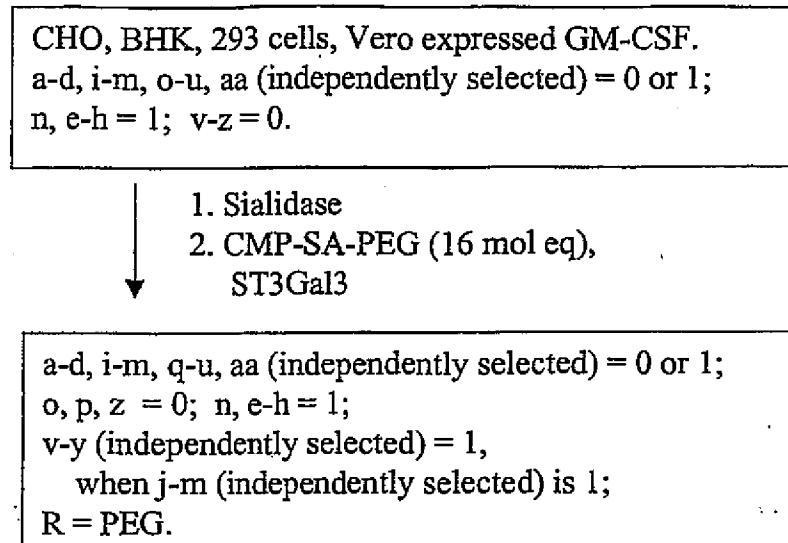


FIG. 36B

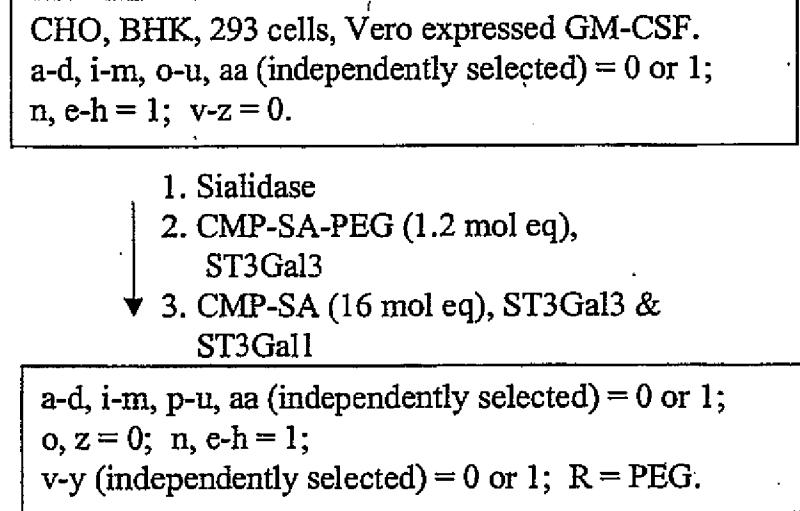


FIG. 36C

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NSO expressed GM-CSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0;  
Sia (independently selected) = Sia or Gal.

- ↓  
1. Sialidase and  $\alpha$ -galactosidase  
2. CMP-SA, ST3Gal3  
2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1;  
n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

## FIG. 36D

CHO, BHK, 293 cells, Vero expressed GM-CSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3  
3. CMP-SA, ST3Gal3

a-d, i-m, q-y, aa (independently selected) = 0 or 1;  
o, p, z = 0; n, e-h = 1; R = PEG.

## FIG. 36E

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CHO, BHK, 293 cells, Vero expressed GM-CSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.

- ↓  
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1;  
z = 0; n, e-h = 1; R = PEG.

FIG. 36F

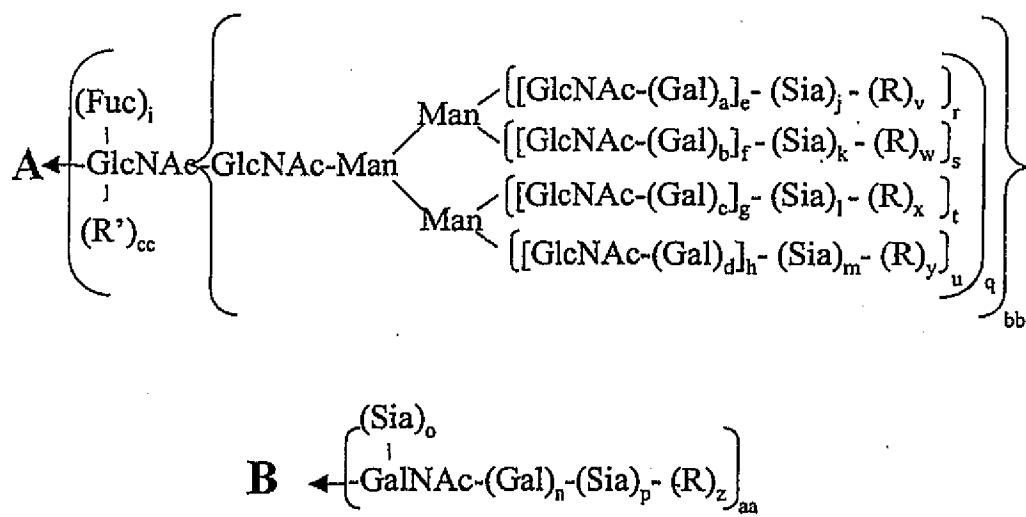
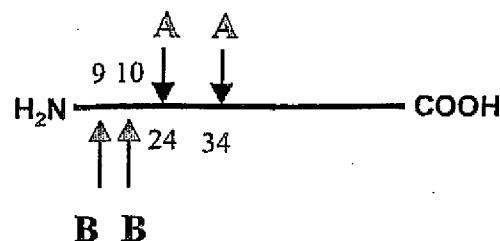
CHO, BHK, 293 cells, Vero expressed GMCSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.

- ↓  
1. CMP-SA, α2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; j-m (independently selected) = 0-20;  
v-z (independently selected) = 0.

FIG. 36G

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a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = modifying group, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group. glycoconjugate.

FIG. 36H

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Insect cell expressed GM-CSF.  
a-d, f, h, j-m, o, p, s, u, v-z = 0;  
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, n, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

FIG. 36I

Yeast expressed GM-CSF.  
a-p, z, cc = 0;  
q-y, aa (independently selected) = 0 to 1;  
bb = 1; R (branched or linear) = Man, oligomannose;  
GalNAc = Man.

- ↓  
1. Endoglycanase  
2. mannosidase (if aa = 1).  
3. Galactosyltransferase, UDP-Gal-PEG

a-p, r-z, aa, bb = 0;  
q, cc (independently selected) = 0 or 1;  
R' = -Gal-PEG.

FIG. 36J

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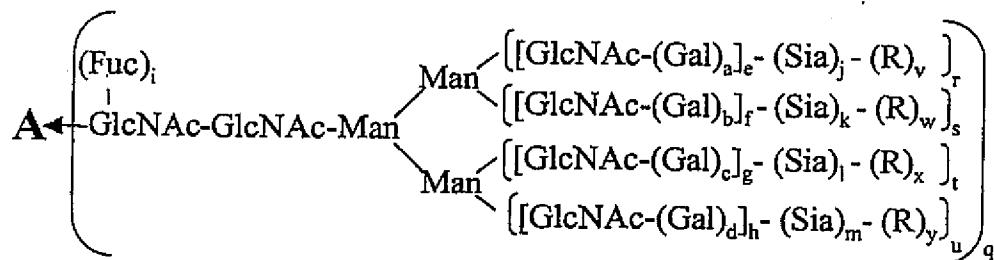
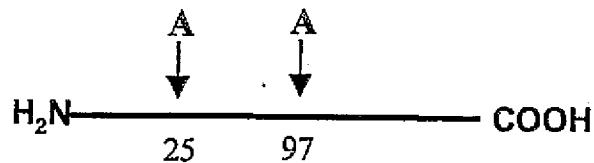
CHO, BHK, 293 cells, Vero expressed GM-CSF.  
a-m, o-u, aa, bb (independently selected) = 0 or 1;  
n, v-z, cc = 0.

- ↓
1. sialidase
  2. CMP-SA, ST3Gal3
  2. CMP-SA-linker-SA-CMP, ST3Gal1
  3. ST3Gal3, transferrin

a--m, p-u, z, aa (independently selected) = 0 or 1;  
o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 36K

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 37A

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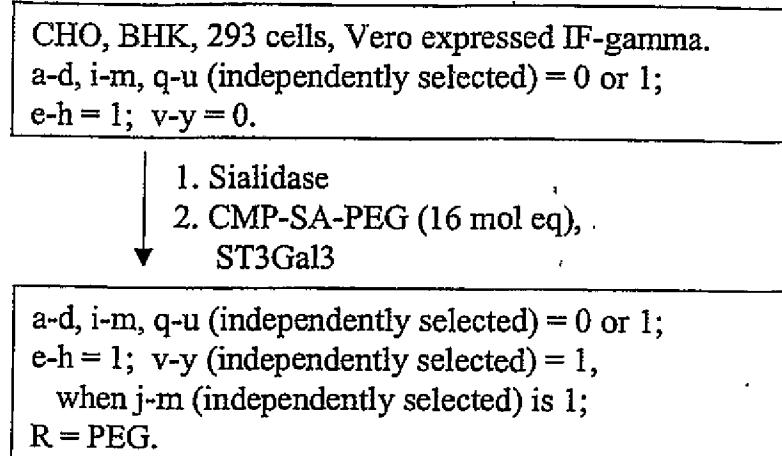


FIG. 37B

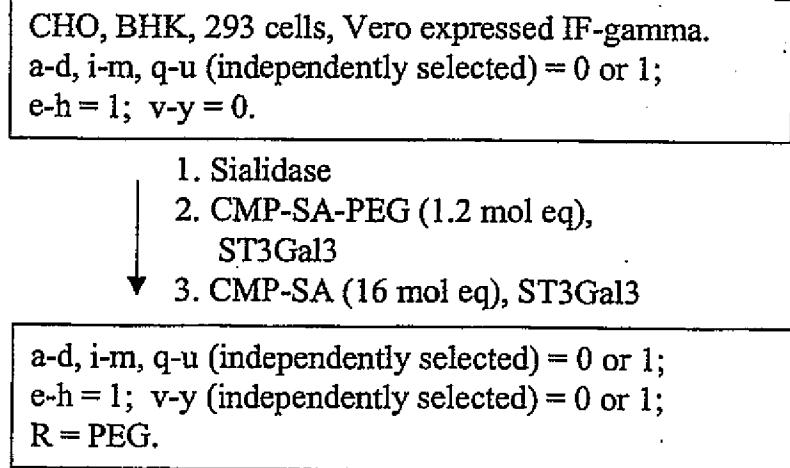


FIG. 37C

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NSO expressed Interferon gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0;  
Sia (independently selected) = Sia or Gal.

- ↓  
1. Sialidase and  $\alpha$ -galactosidase  
2.  $\alpha$ -Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 37D

CHO, BHK, 293 cells, Vero expressed  
Interferon gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3  
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 37E

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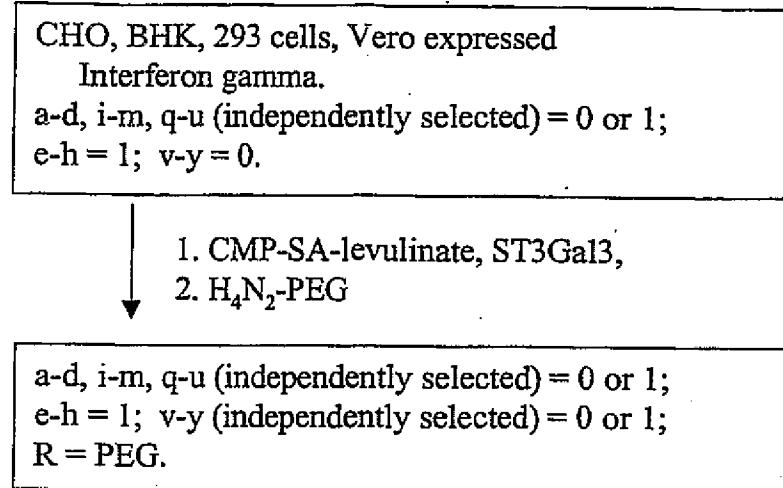


FIG. 37F

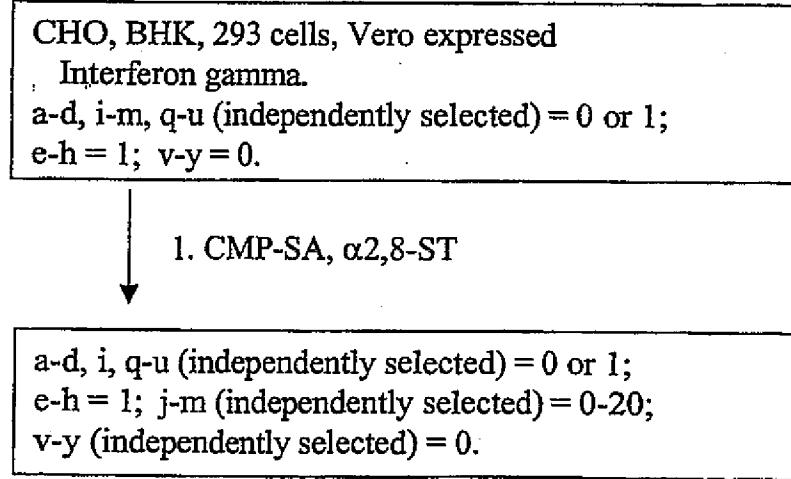
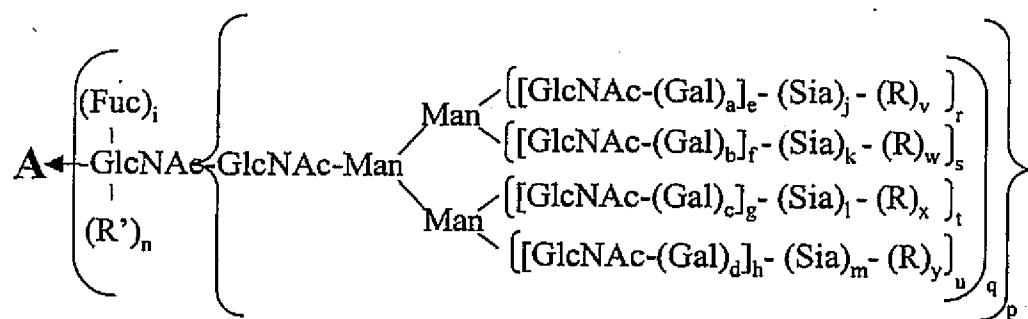
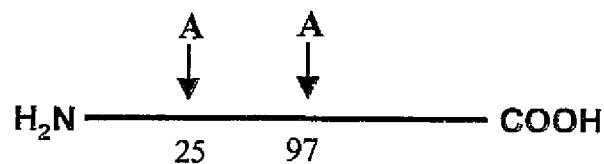


FIG. 37G

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

**FIG. 37H**

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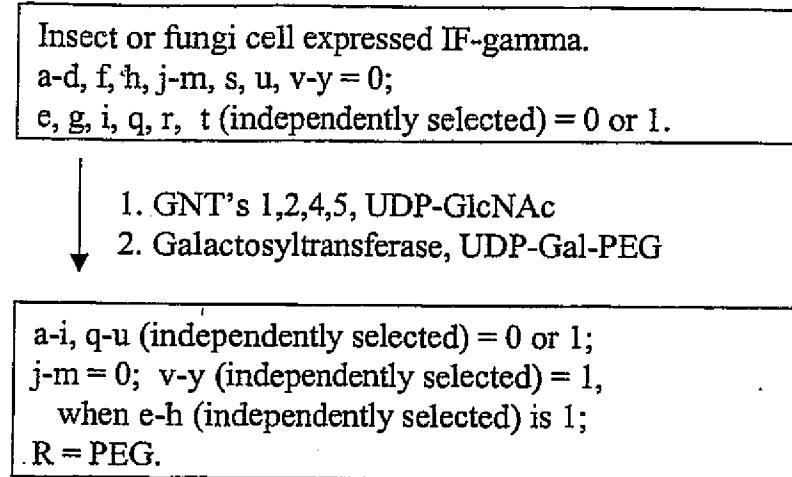


FIG. 37I

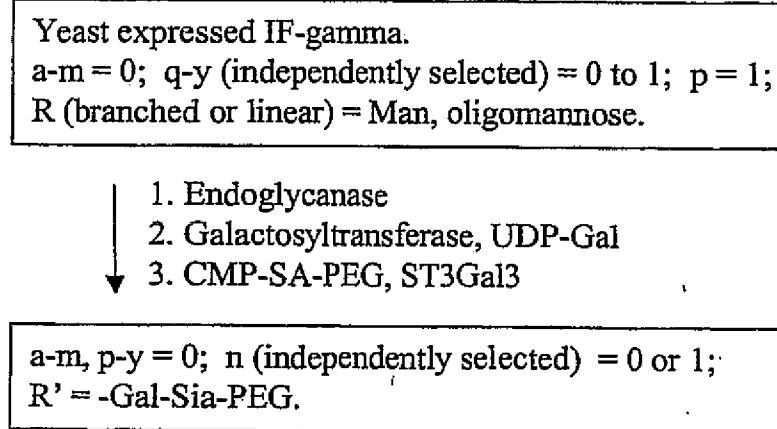


FIG. 37J

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CHO, BHK, 293 cells, Vero expressed IF-gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA-linker-Gal-UDP, ST3Gal3  
2. Galactosyltransferase, transferrin treated  
with endoglycanase.

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 37K

CHO, BHK, 293 cells, Vero expressed  
Interferon gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h, p = 1; n, v-y = 0.

- ↓  
1. CMP-SA-PEG,  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h, p = 1;  
n, v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 37L

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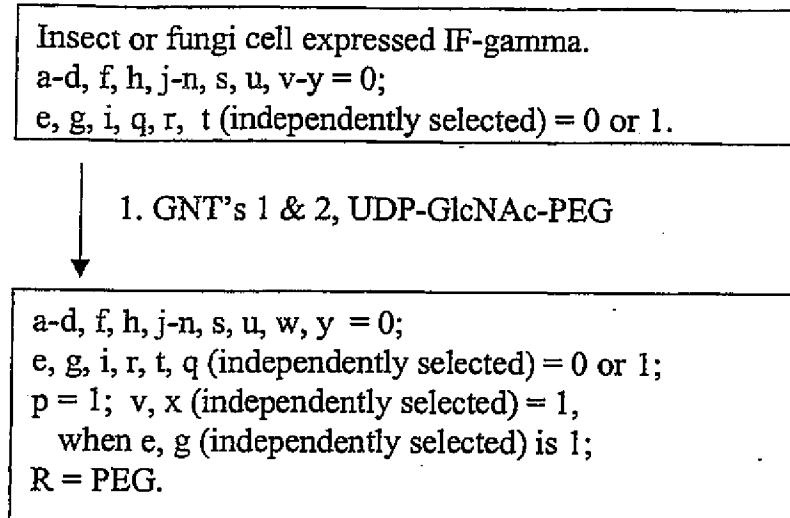


FIG. 37M

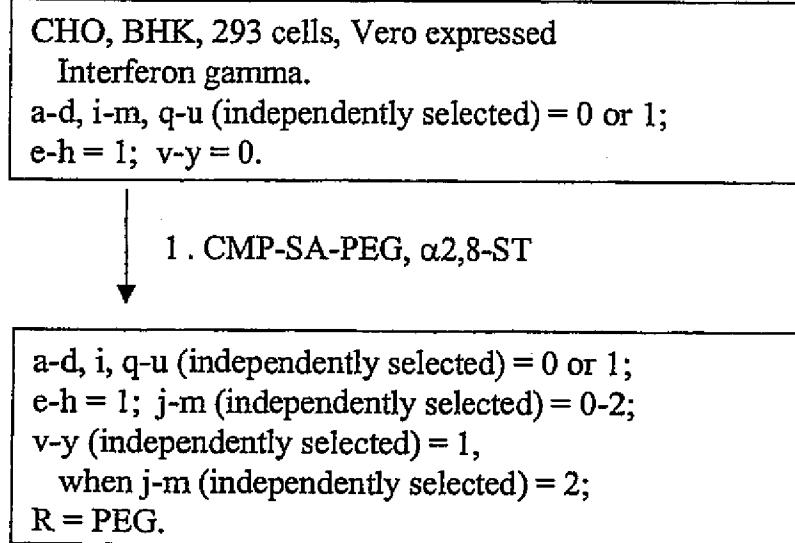
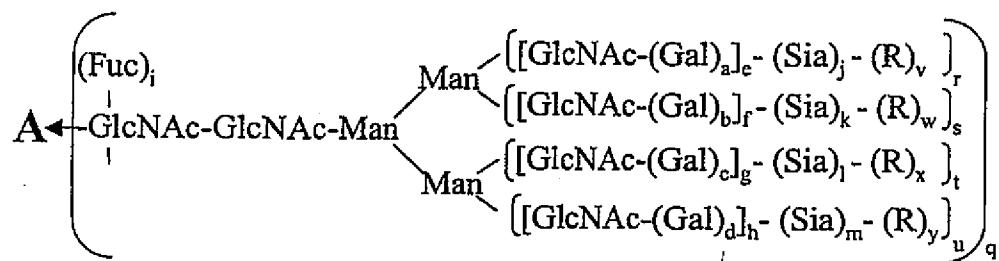
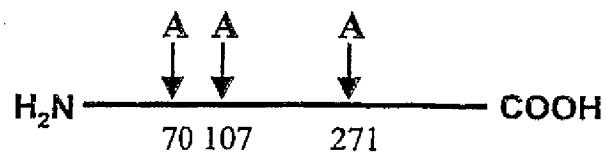


FIG. 37N

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 38A

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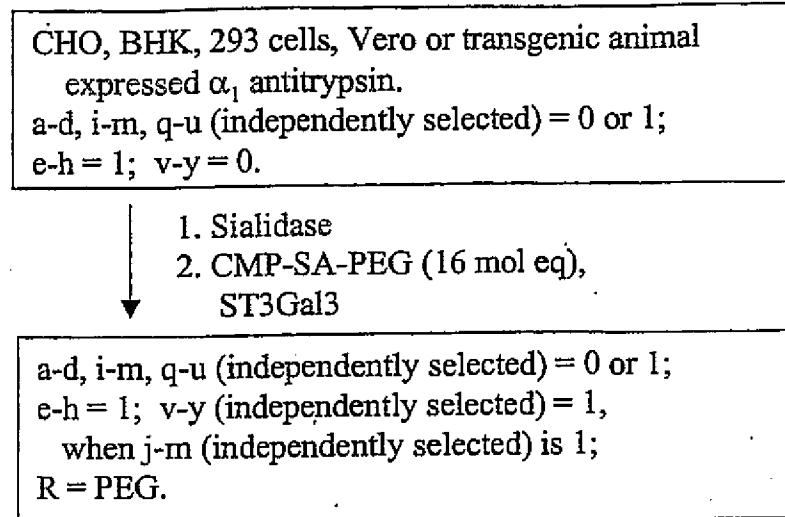


FIG. 38B

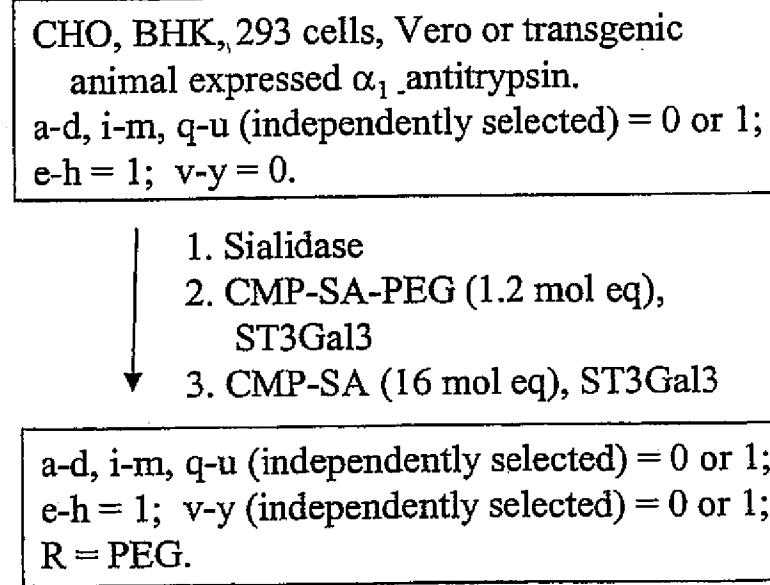


FIG. 38C

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CHO, BHK, 293 cells, Vero or transgenic animal  
expressed alpha-1 antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 38D

CHO, BHK, 293 cells, Vero or transgenic animal  
expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-levulinic acid, ST3Gal3,  
buffer, salt
  2.  $H_4N_2$ -PEG

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 38E

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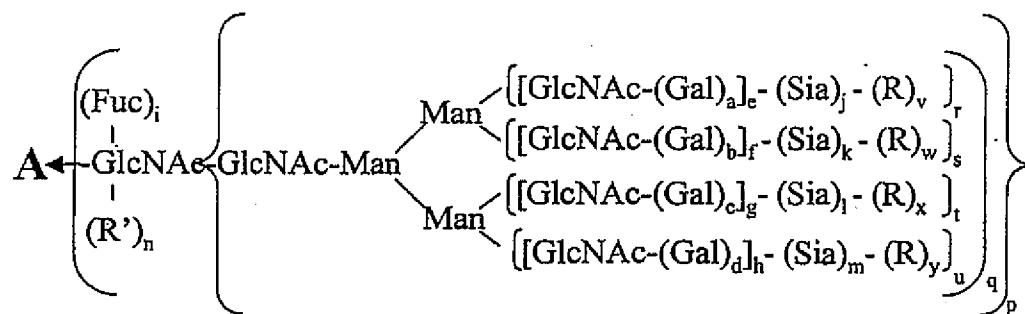
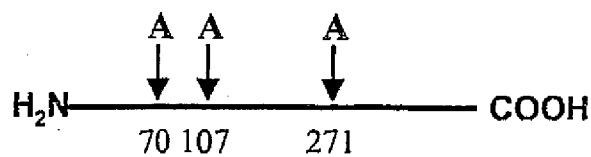
CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

↓  
1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1;  
j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 38F

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 38G

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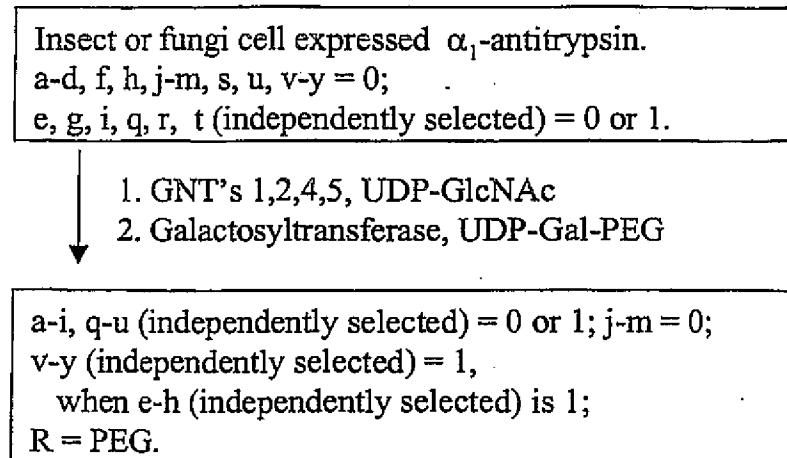


FIG. 38H

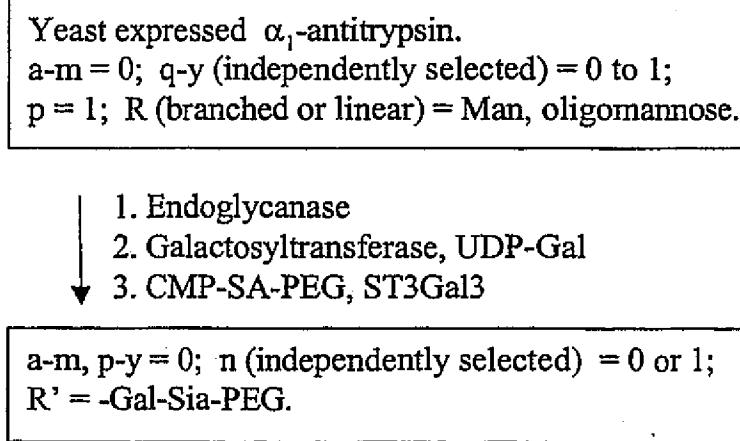


FIG. 38I

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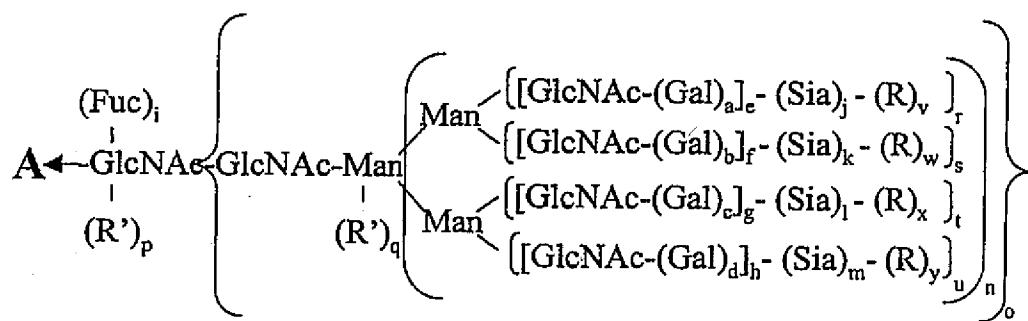
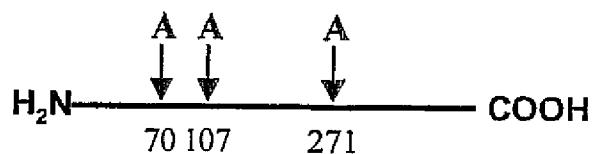
CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

1. CMP-SA-linker-Gal-UDP,  
ST3Gal3
2. Galactosyltransferase, transferrin treated  
with endoglycanase

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 38J

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer;

R', R'' (independently selected) = sugar, glycoconjugate.

**FIG. 38K**

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Yeast expressed alpha-1 antitrypsin.  
a-h, i-m, p, q = 0;  
R (independently selected) = mannose, oligomannose,  
polymannose;  
r-u, v-y (independently selected) = 0 or 1; n, o = 1.

↓  
1. endoglycanase  
↓ 2. Galactosyltransferase, UDP-Gal-PEG

a-h, i-o, q, r-u, v-y = 0; p = 1.  
R'' = Gal-PEG.

## FIG. 38L

Plant expressed alpha-1 antitrypsin.  
a-d, f, h, j- m, s, u , v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1;  
n =1; R' = xylose

↓  
1. hexosaminidase,  
2. alpha mannosidase and xylosidase  
3. GlcNAc transferase, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u , v-y = 0;  
e, g, i, r, t (independently selected) = 0;  
q = 1; R' = GlcNAc-PEG.

## FIG. 38M

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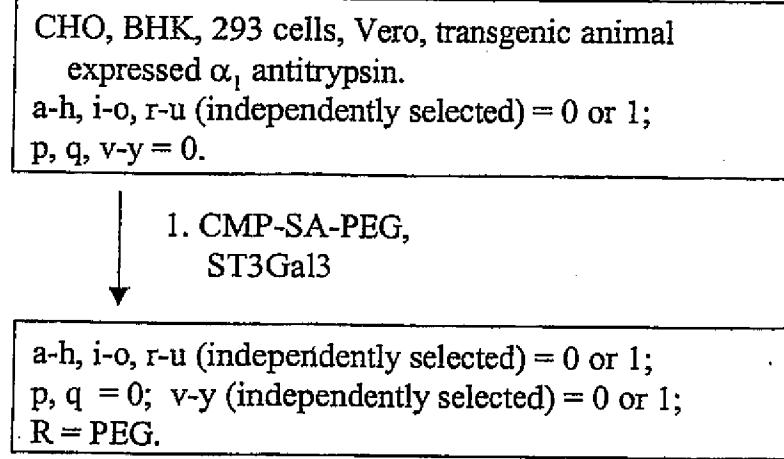


FIG. 38N